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研究課題名（和文）Establishment of an integrated database of DNA repair deficiency disorders

研究課題名（英文）Establishment of an integrated database of DNA repair deficiency disorders

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研究成果の概要（和文）：コケイン症候群関連疾患(CS)は、病態の重篤度から幾つかのグループに分類される。これらの疾患は、遺伝子変異のタイプと病態との関係も明らかになっていない。
本研究にて、収集された１２４例CS症例について、DNA 修復活性の測定とゲノム解析を組み合わせて実施することで、疾患責任遺伝子変異の同定に取り組んだ。まだ、CSB上に共通した疾患原因遺伝子変異を持つ3名のCSタイプIVの患者を得た。CSBに異常のあるCSタイプIとマイルドなCSタイプIVの検体について、患者由来細胞中のCSB蛋白質の発現量や発現状態と紫外線照射後のRNAポリメラーゼの分解反応を調査した。

研究成果の学術的意義や社会的意義
本研究により、これまで明らかになっていなかった、コケイン症候群関連疾患の病態の重篤度の違いを引き起こす原因が明らかになると期待される。また、これらの成果を検討することで、コケイン症候群の発症メカニズムの理解や、TCRでのCSBの機能の解明にも貢献するほか、CSB蛋白質との相互作用因子を調査する過程で、新規TCR関連因子を同定できる可能性も含んでいる。以上のことから、申請者の計画研究は、医学的にも、科学的にも重要な研究と考えられる。。

研究成果の概要（英文）：DNA damage and repair (DDR) system is important for maintenance of genome integrity; defects in DDR can result in several genetic disorders. In current research, we finished diagnosis of 124 patients with Cockyane Syndrome (CS), and identified 71 novel pathogenic mutations in the CSA and CSB genes (J Med Genet, 55 (5): jmedgenet-2017-104877, 2018). We considerably broaden the CSA and CSB mutation spectrum responsible for CS, and improves the definition of the puzzling genotype-phenotype relationships in patients with CS.
We also identified several mild cases with R77* mutations in the CSB gene (CS-IV patients) to search for the reason why different mutations in the CSB genes lead to diverse phenotypes. In our research we performed a series of functional analysis with samples deviated from mild CS-IV and severe CS-I / II patients, to explain the distinct severity of CS clinical phenotype.

研究分野：human genetics

キーワード：Cockayne syndrome　UV-sensitive syndrome　RNA polymerase II

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９、ＣＫ－１９（共通）

１．研究開始当初の背景

DNA damage and repair (DDR) system protects cells from genomic instability, defects in DDR will lead to diverse deleterious outcomes, including cancer, aging and several genetic diseases. Twenty-six diseases are known to associate with DNA repair defects and over fifty genes are identified to be involved. These diseases share common clinical features but with diverse spectrum of symptoms. Overlap of clinical features and complementation groups increase the risk of misdiagnosis.

Previously we have established a diagnostic system for DDR deficiency-related disorders which can provide precise gene diagnosis with limited time and cost. By using a semi-automated, high contents screen (HCS) assay system, along with standard molecular and biochemical methods, and successfully applied the system into analyzing hundreds of DDR patients. During our work in analysis with DDR deficiency disorders, we realized that an integrated database with clinical and genomic information is important, not only for researchers to gain information to understand the underlying mechanisms of complex diseases, but also for clinicians to provide proper clinical diagnosis and for patients to get knowledge and support.

Nucleotide excision repair (NER) is one of the most versatile DDR processes operating in mammals, and is divided into two sub-pathways exist, global genome NER (GG-NER) and transcription-coupled NER (TC-NER). Cockayne syndrome (CS) and UV-sensitive syndrome (UV^{S}S) are two representative genetic disorders associated with defects in TC-NER, both of which show clinical photosensitivity, but only patients with CS also exhibit several developmental and neurological abnormalities. Most CS patients have mutations located in the *ERCC6 (CSB)* and *ERCC8 (CSA)* genes. Interestingly, some specific mutations in the *ERCC6* and *ERCC8* genes were found resulted in UV^{S}S. However, it remains unclear how abnormalities in these proteins lead to diverse clinical symptoms in CS and UV^{S}S.

２．研究の目的

In current research, we are going to collect samples of DDR deficiency patients from all over the world, and aiming to establish an integrated diseases database including clinical features, genomics and proteomics information. We also performed functional analysis to clarify the genotype-phenotype correlations of Cockayne syndrome, as well as the biological importance of RNA Pol II degradation during TC-NER.

３．研究の方法

To build an integrated database of DDR deficiency disorders, we worked on molecular analysis of global collected samples of DDR deficiency patients. We established cell library and performed large scale screening of DNA repair activities, as well as identification of pathogenic genes and mutations involved in DDR deficiency disorders. We also worked on data collection and establishment of an integrated diseases database including clinical features and genomics information.

To understand the underlying mechanisms of CS and UV^{S}S, we collected several samples from CS- IV and CS-I patients, and performed the following functional analysis, including detection of the expression of CSB protein or CSB peptide, as well as analysis of the RNA polymerase II (RNAPII) modification after UV damage.

４．研究成果

Establishment of an integrated database of DDR deficiency disorders

We have collected over 1000 samples and have made the cell stock as well as performed gene diagnosis of most of samples. We, together with collaborators from Italy, France and UK, finished diagnosis of 124 patients with Cockyane Syndrome (CS) (*J Med Genet*, 55 (5)：jmedgenet-2017-104877, 2018). Out of the 124 patients identified as having a specific defect in RRS, 39 were mutated in *ERCC8/CSA* and 85 in *ERCC6/CSB*, respectively. We considerably broaden the *CSA* and *CSB* mutation spectrum responsible for CS by identifying 71 novel homozygous or compound heterozygous genetic variants with different disease severity and ethnic backgrounds. Although no definitive correlations between genotype and phenotype was confirmed in the research, our data strongly suggest that type II features were more prevalent in patients with CS-B than in patients with CS-A. We also identified a founder *CSA* mutation in Japanese CS patients. In a word, besides providing information relevant for diagnosis of and genetic counselling for this devastating disorder, this study improves the definition of the puzzling genotype-phenotype relationships in patients with CS.

Functional analysis of CS and UV^{S}S patients

CSB is a 1493-aa protein containing seven helicase-like ATPase motifs (Gool, 1992) and an ubiquitin-binding domain in the C-terminus (Anindya, 2010, Mol Cell). Previously Weiner's group discovered a PGBD3 transposon (piggyBac transposable element-derived 3) that located in the intron 5 of CSB gene, which generate a 1061- aa CSB-PGBD3 fusion protein containing CSB exons 2-5 and PGBD3 transposase (Newman, 2008, PLoS Genet; Gray, 2012, PLoS Genet). They hypothesized that nonsense mutation upstream CSB exon 5 would generate neither protein, thus lead to mild UV^{S}S phenotype; while downstream mutations generating no full-length functional CSB protein but CSB-PGBD3 and/or CSB truncation would lead to severe CS phenotype.

In our research, we identified three Japanese mild late-onset CS-type IV / UV^{S}S cases with homozygous or compound heterozygous mutations consisting of the Arg77* in the *CSB* gene. We checked expression of CSB protein by immunoblotting in normal individual as well as CS-I, CS-IV and UV^{S}S patients-deviated cell lines. We found that tiny amount of the CSB missense mutation expressed in a CS-IV patients, which was also identified in a severe CS-II / COFS Japanese case. Inconsistent with the former hypothesis, our result revealed that expression of dysfunctional CSB and CSB-PGBD3 may also lead to mild UV^{S}S / CS-IV phenotype. Moreover, we found similar protein expression pattern with expression of CSB-PGBD3 and undetectable full- length CSB in both CS-I and CS-IV deviated samples, which further suggested that severity of CS phenotype might be not related to the expression of CSB-PGBD3.

In the further research, we will focus on investigate the different molecular response in CS-I and CS-IV patients, including RNAPII modification and expression of CSB peptide or alternative transcripts.

５．主な発表論文等

〔雑誌論文〕（計２件）*equal contribution

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〔図書〕（計０件）
なし

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〔その他〕
ホームページ等
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